CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

University of Michigan, Ann Arbor, Michigan

Number 35 May, 1947

AN ANALYSIS OF CERTAIN GENETIC VARIATIONS IN PELAGE COLOR OF THE CHIHUAHUA DEER-MOUSE (*PEROMYSCUS MANICULATUS BLANDUS*)

By W. Frank Blair

Accepted for publication July 19, 1946

CONTENTS

Introduction 1
Acknowledgments 2
Methods 2
Color Strains 4
Mode of Inheritance of Major Color Characters 10
Variations Within Major Color Classes 13
Discussion 15
Summary 17
Literature Cited 17

INTRODUCTION

Genetic analysis of the differences between wild-type individuals comprising local populations, races, and species of animals is a necessary step toward understanding the complex process of evolution of animal kinds. Laboratory studies of mutant characters must be extended to include the attributes that differentiate between free individuals and taxonomic groups if genetics is to make its most effective contribution toward solving the problems of evolution.

Wild genotypes are more difficult to analyze genetically than are the usual laboratory-bred strains of animals. The wild genotype is a complex system of major and minor genes that has been molded into attunement with its environment by the forces of mutation pressure, gene dispersal, and local selection pressures. The effects of major genes in wild populations consequently may be obscured by the accumulated effects of other minor, modifying genes. The obvious method of attack is to bring the animals into the laboratory and there to establish homogeneous strains that exhibit the characters of the wild populations. This is the approach used in the present undertaking.

This report deals with genetic analysis of certain wild-type variations in pelage color of the deer-mouse (*Peromyscus maniculatus blandus*). This particular animal was chosen because the variable deer-mouse populations of the southwestern deserts provide the best material for such a study. Even small local populations in the desert contain mice of sharply different hues and shades of color. The magnitude and relative discontinuity of these differences suggest the possibility of single gene differences in major color characters.

The stock of deer-mice treated here came from the Tularosa Basin of southern New Mexico. Buff and gray color phases were recognizable in the original sample of the wild population. These buff and gray mice were used to establish laboratory populations of the corresponding color types. The number of color strains was later increased as other color differences were recognized. The several stocks were then crossed to determine the mode of inheritance of the different color characters.

The buff-gray dimorphism of deer-mice has received some attention in the past. Osgood (1909) observed buff and gray forms of deer-mice in four different geographic races. Collins (1923) showed that the differences between buff and gray are genetic. Dice (1933) crossed buff and gray deer-mice and showed that buff and gray are alternative characters of which buff is dominant over gray. The present study further tests the mode of inheritance of buff and gray coat color and also deals with other variations in pelage color of these deer-mice.

ACKNOWLEDGMENTS

This study was conducted at the Laboratory of Vertebrate Biology, University of Michigan. The field trip to collect the parent stock of mice was supported by a grant to Lee R. Dice from the Carnegie Institution of Washington. I am indebted to Lee R. Dice and Elizabeth Barto for critically reading the manuscript and to Miss Barto for kindly making available certain unpublished data.

METHODS

The parent stock of deer-mice was trapped alive about three miles north of Tularosa, Otero County, New Mexico, from October to December, 1938. The field-caught sample shipped alive to the Laboratory of Vertebrate Biology comprised 108 deer-mice. These mice and their offspring were kept under standard laboratory conditions and were fed a standard ration. The offspring of the original, field-caught mice were used to establish the color strains. Inbreeding was held to a minimum during selection within the color stocks, for even a few generations of close inbreeding produces a high degree of infertility in *Peromyscus*. As a rule, any intra-sibship matings were followed by inter-sibship matings between similar phenotypes as approximated by visual inspection.

The offspring of crosses between the color strains were classified as to color type while the mice were alive. Then the mice were prepared as flat skins at an age of approximately five months. The mice were classified again into color types after the skins were prepared. No important difference was found in the color identifications of the living mice and the prepared skins.

Gross color analyses of the several strains and the offspring of the crosses have been made with a Photovolt photoelectric reflection meter, model 610. This instrument consists of a galvanometer and a search unit. The light source is a six to eight volt, fifteen candle-power headlight bulb. The light reflected from the chosen area of the mouse skin stimulates a photoelectric cell. The electric current produced by this cell, when stimulated by reflected light, is read on a sensitive galvanometer. The search unit is placed on a block of white magnesium carbonate, and the galvanometer needle set at 100 before each reading on a skin. The zero point is standardized by projecting the light from the search unit into a tube of dull black paper. The galvanometer reading, then, is the percentage of light reflected from the skin as compared to the amount reflected from the magnesium block. The aperture of the search unit is masked to pick up the light reflected from an area of 9.3 by 7.5 mm. All reasonable precautions have been taken to eliminate stray light and to prevent mechanical error.

Color readings have been made routinely on the dorsal stripe of the flat skins in approximately the middle of the back. For some comparisons readings also have been made on the colored area of the side. A red filter was used alone for readings on the dorsal stripe when the comparisons between stocks principally involved differences in shade and saturation. When a measure of the qualitative differences between stocks was desired, red, green, and blue filters were used for both mid-dorsal and side readings. All color readings have been made by the author or by an assistant, William Jalosky. Repetition of random samples of the other's work by each of the operators indicates the reliability of this method of color measurement.

The reflection-meter values are not directly comparable with color measurements made with the Ives tint photometer originally applied to mammalian colorimetric work by Sumner (1927) and subsequently used in many studies of mammalian pelage color (Dice, 1932). The greater objectivity and ease of operation of the reflection meter make its use highly preferable to use of the tint photometer.

No attempt has been made to analyze the minute details of the hair structure and pigmentation of the color strains. Sample hairs from the several stocks have been examined, however, to determine their major differences in pigmentation. Hairs have been taken for examination from the hip region, the dorsal stripe in the lumbar region, and from the vicinity of the root of the tail of sample dried skins. The hairs were soaked overnight in xylene to eliminate air spaces, then they were mounted in clarite for microscopic examination. The major details of the hair pigmentation agree with those described by Collins (1923), with one exception mentioned below.

Only two types of pigments are present in the deer-mouse hairs, but each of these varies greatly in its intensity. The yellow-orange (yellow of Collins) pigment ranges from pale lemon yellow to intense yellow-orange. The sepia pigment varies from pale brown in some stocks to black in others. My observations agree in part with those of Collins (1923) that the intensity of the pigment appears to be proportionate to the size of the granules and the density of the masses in which they occur. The granules of pale sepia are possibly also qualitatively paler than are the dark sepia granules. I do not agree with Collins (1923) that the yellow-orange occurs in both granular and diffuse form, as this pigment appears granular in our material. Collins' observation of diffuse pigment possibly results from his use of glycerin as a mounting medium. It has been recently observed by Russell (1946) that glycerin sometimes reacts with yellow pigment in the house mouse (*Mus musculus*). This leads to a gradual breakdown of the yellow granules, leaving the cells filled with diffuse yellow coloration.

The chi-square test has been used to determine the significance of deviations from the expected ratios in F_2 and backcross generations. Comparisons between means have been made by the method of Dice and Leraas (1936). Differences between means which are greater than 2.7 times the standard error of their difference are considered significant.

COLOR STRAINS

Five selection lines were established by using the offspring of the field-caught deer-mice. These lines represent four major phenotypes. The fifth line is accounted for by selection in opposite directions (that is, toward paleness and darkness of pelage) within the limits of one of the major color types. All lines eventually bred true to type except for minor variations in saturation and brilliance of color. The color strains are described as follows:

Black-tipped buff. The dark buff mice of this strain do not differ in appearance from dark buff mice in the original sample of the wild population. In gross appearance, the dorsal pelage is a dark, reddish buff liberally sprinkled with black or dark brown guard hairs. The tail stripe is sharply defined and intensely black. Selection was attempted toward an increase in the saturation of the buff color, but the saturation of some of the field-caught mice never was exceeded. The juvenile pelage usually is distinctly buffy, and many of the young can be identified as soon as the hair appears.

In shade of pelage color, as shown by reflection-meter readings for mid-dorsal red, the black-tipped buff strain is darker than any except one strain of black-tipped gray (Table I). The readings for reflected red on the dorsal stripe among ninety-four specimens prepared at random from the maintenance stock average 14.86 ± 0.25 reflection-meter units. This is slightly, but significantly, darker than the mean of eighty-two field-caught buffs, for the average of the field-caught mice is 16.12 ± 0.27 units, and the difference in the means is 1.26 ± 0.36 units.

TABLE I

SUMMARY OF PELAGE CHARACTERS OF COLOR STRAINS

Strain	Gross Appearance of Pelage	Shade of Pelage Reflection-Meter Readings (Reflected Red)	Characters of Hairs		
			Body (Agouti) Hairs		Guard (Nonagouti) Hairs
			Proximal Band	Subterminal Band	
Black-tipped buff	Dark buff. Tail stripe black, sharply defined	14.86 ± 0.25	Large, black clumps sepia pigment	Intense yellow-orange pigment; scattered granules of sepia	Dark brown or black cortical pigment at tip; black masses of sepia throughout
Black-tipped gray (dark)	Dark gray. Tail stripe black, sharply defined	12.85 ± 0.32	Large, black clumps sepia pigment	No discernible yellow-orange pigment; granules or small clumps of sepia	Dark brown or black cortical pigment at tip; black masses of sepia throughout
Black-tipped gray (pale)	Pale gray. Tail stripe gray to black, not sharply defined	21.60 ± 0.24	Moderately large, black clumps sepia pigment	No discernible yellow-orange pigment; granules or small clumps of sepia	Dark brown or black cortical pigment at tip; black masses of sepia throughout
Brown-tipped buff	Yellow. Tail stripe pale brown	34.15 ± 0.23	Small, brown, granular clumps sepia pigment	Pale yellow-orange pigment; scattered granules of sepia	Pale brown cortical pigment at tip; small, brown clumps of sepia throughout
Brown-tipped gray	Pale brown. Tail stripe pale brown	30.57 ± 0.33	Small, brown, granular clumps sepia pigment	No discernible yellow-orange pigment; scattered granules of sepia	Pale brown cortical pigment at tip; small, brown clumps of sepia throughout

The hair pigments of the black-tipped buff mice are intensely dark, as would be expected from the gross appearance of the pelage. The proximal band of the body, or agouti, hairs contains large, black clumps of sepia pigment. The subterminal band has intense yellow-orange pigment and scattered granules of sepia. The sepia pigment in the cortex of the terminal band varies from dark brown to black in appearance. The guard, nonagouti, hairs have black masses of sepia throughout their length, and the cortical pigment at the tip is dark brown or black.

Black-tipped gray (dark selection line). The dark gray mice of this strain do not differ in appearance from gray field-caught mice except for their greater darkness. The dark gray pelage has a somewhat buffy-brown tone, and close observation reveals numerous black or dark brown guard hairs. The tail stripe is intensely black and sharply defined, as in the black-tipped buffs. Selection in this line was toward darkening the shade of pelage color and eliminating the buffy-brown tone.

The mice of this strain are darker than those of any other line. The readings for reflected red on the mid-dorsal stripe among forty-one individuals taken at random from the maintenance stock average 12.85 ± 0.32 reflection-meter units (Table I). These mice average slightly, but significantly, darker than the black-tipped buff mice, for the difference between their means is 2.01 ± 0.40 units. This gray strain averages darker than the field-caught group of seventeen grays. The field-caught grays average 16.47 ± 0.54 units, and the difference between the two means is 3.61 ± 0.63 units.

These mice differ principally from black-tipped buffs in lacking discernible yellow-orange in the subterminal band of the body hairs. Instead of yellow-orange pigment, the subterminal band in the gray mice contains scattered granules or small masses of sepia. The buffy-brown tone of the black-tipped grays appears to result from the small masses and granules of sepia in the subterminal band. Small quantities or loose clumps of sepia appear brown under the microscope regardless of where they occur. The pigmentation of the proximal and terminal bands of the agouti hairs and the pigmentation of the guard hairs do not differ from that of the corresponding bands in black-tipped buff mice.

Black-tipped gray (pale selection line). In these pale gray mice, as in the preceding stocks, the guard hairs of the dorsal surface appear black or dark brown. The tail stripe is less intensely black and less sharply defined than in the black-tipped buffs and black-tipped dark grays. This pale stock was selected toward increased brilliance of gray. Selection was effected to the point at which the shade of pelage color of the darkest mice of this strain barely overlapped the range of the palest mice of the dark gray strain.

The average shade of pelage color in this strain is shown by reflection-meter readings for mid-dorsal red on eighty mice from the maintenance stock (Table I). The mean is 21.60 ± 0.24 reflection-meter units. These mice are considerably paler than the field-caught grays from which the line originated, for the difference in the means of the two groups is 5.13 ± 0.59 units.

This strain, like the dark gray line, differs from buff mice in lacking any discernible yellow-orange pigment in the subterminal band of the body hairs. The pale gray appear to differ from the dark gray mice in a general, although fairly slight, reduction of the size and density of the masses of sepia pigment. The subterminal band on the agouti hairs of the pale gray mice contains fewer granules of sepia and fewer and smaller clumps of the same pigment.

Brown-tipped buff. These mice are pale buff or yellow-orange in gross appearance. The tips of the guard hairs are pale brown, and these pale tips contrast only slightly with the ground color contributed by the body hairs. The result is a pelage that is much more uniform in color than is the pelage of the black-tipped buffs and grays. The tail stripe is pale brown and indistinctly defined. In juvenile pelage these mice are pale buff, and the young can be distinguished from the young of black-tipped mice as soon as the hair appears.

The original brown-tipped buff mice appeared as segregants in routine crosses between black-tipped buffs. The ancestry of these first brown-tipped buffs traces back to four field-caught, black-tipped buff mice, but the pedigrees do not show how many of the field-caught mice were heterozygous for brown-tipped. Little selection was made in the brown-tipped buff strain. The stock was maintained at a moderate intensity of yellow-orange, although occasional variants both above and below this intensity would have permitted some selection in either direction.

In shade of pelage color the brown-tipped buff is the palest of all the strains. Reflection-meter readings for reflected red on the mid-dorsal surface of 104 mice from the maintenance stock average 34.15 ± 0.23 units. The darkest of these is much paler than the palest of the field-caught mice. The palest field-caught mouse, a black-tipped buff, gives a reflection-meter reading of only twenty-one units; the darkest of the brown-tipped buffs gives a reading of twenty-nine units.

In the details of hair pigmentation, the brown-tipped buff mice differ greatly from the strains described above. Like the black-tipped buffs, the brown-tipped buffs have conspicuous yellow-orange pigment in the subterminal band of the body hairs. This pigment, however, is lemon yellow in contrast to the intense yellow-orange of the black-tipped buffs. The chief difference between the brown-tipped and black-tipped strains is in the density and size of the clumps of sepia pigment in all of the pigmented hairs. In the brown-tipped mice the clumps of sepia are smaller, and the individual granules making up each clump are less compacted than they are in the black-tipped mice. The loosely gathered granules comprising a clump are separately identifiable, and as a result each clump is extremely granular in appearance. The loose arrangement of the sepia granules produces a pale brown color, which contrasts with the black or dark brown of black-tipped mice. This pale brown is found in both the medulla and cortex of the hairs of brown-tipped mice. The pale brown tips of the guard hairs, as seen with the naked eye, result from the paleness of the sepia pigment in the cortex.

Brown-tipped gray. These mice are pale brown in general appearance. The coat of hair is relatively uniform in color, as is the case in brown-tipped buffs. The tail stripe is pale brown and indistinctly defined. The juvenile pelage is pale brown, and these mice, like the brown-tipped buffs, can be identified as soon as the hair grows out.

The original mice of this strain appeared as segregants in two pairs of matings between mice of the black-tipped, pale-gray line. Others were obtained by crossing brown-tipped buffs with black-tipped, pale gray mice and removing the brown-tipped grays in the F_2 generation. The brown-tipped grays trace back to the same field-caught ancestors as the brown-tipped buffs. One brown-tipped gray mouse was trapped in the wild. This was an immature mouse about three weeks of age, which died in the trap and was preserved as a flat skin. The brown-tipped gray line was rigorously selected for paleness.

Some measure of the qualitative color differences between brown-tipped gray and brown-tipped buff is afforded by reflection-meter readings with three color filters, red, green, and blue (Table II). The buffs had significantly higher values for red, both mid-dorsal and side, than did the grays. The gray mice, on the other hand, gave significantly higher readings for green and blue than did the buffs. The greatest difference between the two strains is with reflected blue from the side, where the difference in the means is 5.89 ± 0.33 reflection-meter units. The brown-tipped buff mice, then, are relatively high in reflectance of light rays falling in the red band of the spectrum and low in reflectance of greens and blues. The brown-tipped grays are relatively low in reflectance of red rays and high in reflectance of greens and blues. This is what one would expect from the gross appearance of the pelage of the two strains.

The pigmentation of the hairs in this strain is the same as in brown-tipped buffs with one important exception (Table I). The subterminal band of the agouti hairs, as in the black-tipped grays, lacks discernible yellow-orange pigment. Granules of sepia pigment are scattered through the subterminal band of the body hairs. The sepia pigment of the brown-tipped grays is pale brown, like the sepia pigment of brown-tipped buffs.

Brown-tipped buff and gray mice of my strains have been compared with specimens of recessive "brown agouti" deer-mice described by Huestis and Barto (1934) from a stock of *Peromyscus maniculatus gambelii* originating at Silver Lake, Oregon, and both strains are alike in their pale brown sepia pigments. Crosses made by Elizabeth Barto (unpublished) between my

TABLE II

Reflection-Meter Readings on Brown-Tipped Buff and Gray Mice of Selection Line Stocks

Means, Standard Errors and Differences

Line	Mid-Dorsal			Side		
	Red	Green	Blue	Red	Green	Blue
Brown-tipped Buff	34.15 ± 0.23	18.55 ± 0.16	11.89 ± 0.14	45.50 ± 0.27	27.50 ± 0.24	18.11 ± 0.19
Brown-tipped Gray	30.57 ± 0.33	19.59 ± 0.20	14.74 ± 0.20	42.01 ± 0.34	30.19 ± 0.28	24.00 ± 0.27
Differences of Gray from Buff	− 3.58 ± 0.40	+ 1.04 ± 0.26	+ 2.85 ± 0.24	− 3.49 ± 0.43	+ 2.69 ± 0.37	+ 5.89 ± 0.33

"brown-tipped gray" from Tularosa and "brown agouti" mice descended from those from Silver Lake produced an F_1 generation of twenty-nine brown-tipped mice and an F_2 generation of thirty-eight brown-tipped mice. The characters "brown-tipped" and "brown agouti," therefore, appear to be produced by the same gene or by alleles so similar that no difference can be recognized. The original "brown agouti" was, from its description, a brown-tipped buff of my nomenclature.

MODE OF INHERITANCE OF MAJOR COLOR CHARACTERS

Cross matings between the stocks were made in various combinations to determine the mode of inheritance of the distinctive color characters of these strains. The major differences between buff and gray deer-mice already have been shown to be due to the action of a single pair of alleles (Dice, 1933). These genes have been designated agouti buff (A^b) and gray (A) by Clark (1938). As it is unlikely that these genes are homologous with the agouti genes of the house mouse, it seems preferable to designate the deer-mouse genes by the symbols (G) and (g), respectively. My recessive strains, brown-tipped buff and brown-tipped gray, appeared to be parallel, pale modifications of buff and gray. It is possible, but by no means certain, that black-tipped and brown-tipped are homologous of black and brown of the house mouse. On the assumption that they are homologous they are here given the symbols (B) for black-tipped and (b) for brown-tipped. The cross matings were designed, therefore, to test the hypothesis that the major color differences in the Tularosa stock are produced by two pairs of alleles. The results of the crosses between my color strains are summarized in Table III.

Brown-tipped gray, when crossed with brown-tipped buff, produced brown-tipped buff offspring with the exception of one brown-tipped gray, which indicates that one buff parent was heterozygous for buff-gray. Matings between the buff F_1 mice resulted in an F_2 generation of sixty-four brown-tipped buff and twenty-eight brown-tipped gray mice. The frequencies of the two phenotypes showed no significant deviation from the expected frequencies in a cross involving a single pair of alleles (Table III). Some of the F_1 progeny were backcrossed to brown-tipped gray. The offspring of this backcross comprised sixty brown-tipped buffs and forty-nine brown-tipped grays. The frequencies of the two classes showed no significant deviation from the expected 1:1 ratio for a simple backcross. This is evidence that brown-tipped buff and brown-tipped gray are homozygous for the brown-tipped gene and that buff is a single unit dominant over gray.

When brown-tipped gray was crossed with black-tipped dark gray, an all black-tipped gray F_1 generation was produced. The F_1 mice were somewhat paler than their black-tipped gray parents, but there was no doubt about their being black-tipped rather than brown-tipped. This effect on shade of color in all crosses between black-tipped and brown-tipped mice will

TABLE III

Segregation in Crosses Between Black-Tipped (B) and Brown-Tipped (b) and Buff (G) and Gray (g) Strains of Deer-Mice (*Peromyscus maniculatus blandus*)

Crosses	F₁ Expected No.	F₁ Phenotypes	F₁ Observed No.	F₂ Expected No.	F₂ Classes of Phenotypes	F₂ Observed No.	χ^2
Brown-tipped gray				69.00	*b G*	64	
×	16	*b G*	15				1.149
Brown-tipped buff		*b g*	1*	23.00	*b g*	28	
Brown-tipped gray				81.00	*B g*	80	
×							0.049
Black-tipped gray (dark)	46	*B g*	46	27.00	*b g*	28	
Brown-tipped buff	61	*B G*	60	48.00	*B G*	49	
×							0.083
Black-tipped buff		*B g*	1*	16.00	*b G*	15	
Brown-tipped buff				105.00	*B G* and *B g*	115	
×							3.809
Black-tipped buff (heterozygous for gray included)				35.00	*b G* and *b g*	25	
Brown-tipped gray				114.75	*B G*	110	Interaction
×				38.25	*B g*	44	
Black-tipped buff	40	*B G*	40	38.25	*b G*	38	1.281
				12.75	*b g*	12	
Brown-tipped buff	20	*B G*	19	54.00	*B G*	50	Interaction
×				18.00	*B g*	20	
Black-tipped gray (dark)		*B g*	1*	18.00	*b G*	21	2.722
				6.00	*b g*	5	
Black-tipped buff				9.25	*B G*	11	
×	22	*B G*	23	18.50	*B G*†	17	
				9.25	*B g*	9	
Black-tipped gray (dark)		*B g*	9*				

BACKCROSS

Crosses	Expected No.	Classes of Phenotypes	Observed No.	χ^2
F₁ (brown-tipped gray × brown-tipped buff)	54.50	*b G*	60	
×			49	1.111
brown-tipped gray	54.50	*b g*		

---

* Due to some heterozygosis in parental stocks; not used to produce F₂, nor backcrosses.

† Difficult to classify; possibly heterozygotes.

be discussed later. The F_2 generation contained eighty black-tipped grays and twenty-eight brown-tipped grays. This ratio does not depart significantly from the expected 3:1 ratio in a cross involving a single pair of Mendelian alleles (Table III). It seems evident that black-tipped and brown-tipped gray are homozygous for recessive gray and that black-tipped is dominant over brown-tipped.

The crossing of brown-tipped buff with black-tipped buff produced black-tipped buff offspring with the exception of one black-tipped gray. Since the same level of homozygosity for buff had not been obtained in these strains as in those used in other crosses, the F_2 generation contained some black-tipped and brown-tipped grays. In some sibships only buffs appeared. The frequencies of forty-nine black-tipped and fifteen brown-tipped buffs in this part of the F_2 generation were close to the expected 3:1 ratio for a simple Mendelian cross (Table III). In the total F_2 group the frequencies of 115 black-tipped (including both buffs and grays) and twenty-five brown-tipped (buffs and grays) deviate somewhat from the expected 3:1 ratio. However, this deviation is not considered significant, as the chi-square value is only 3.809 with one degree of freedom. The results of this cross show that black-tipped buff and brown-tipped buff have the gene for buff in common and that black-tipped is dominant over brown-tipped.

TABLE IV

TEST FOR LINKAGE BETWEEN BUFF—GRAY ($G\ g$) AND BLACK-TIPPED—BROWN-TIPPED ($B\ b$)

Test-cross
($B\ G/b\ g \times b\ g/b\ g$)

Class	Observed	Expected
Black-tipped, buff	66	59.25
Black-tipped, gray	63	59.25
Brown-tipped, buff	54	59.25
Brown-tipped, gray	54	59.25
Total	237	237.00

Analysis of Chi-square

Source of Variation	Degrees of Freedom	Chi-square
Black-tipped: brown-tipped ratio	1	1.861
Buff: gray ratio	1	0.038
Linkage	1	0.038
Total	3	1.937

Conclusive evidence that the major characters of the color strains result from the action of two pairs of alleles is found in the cross between brown-tipped buff and black-tipped gray. All F_1 progeny were black-tipped buff except for one black-tipped gray assumed to be indicative of a heterozygous parent. Black-tipped buff is a recombination class here, as the gene for black-tipped must have come from one parent and the gene for buff from the other. The black-tipped buff F_1 produced an F_2 generation of fifty black-tipped buffs, twenty black-tipped grays, twenty-one brown-tipped buffs, and five brown-tipped grays. The frequencies of these phenotypes in

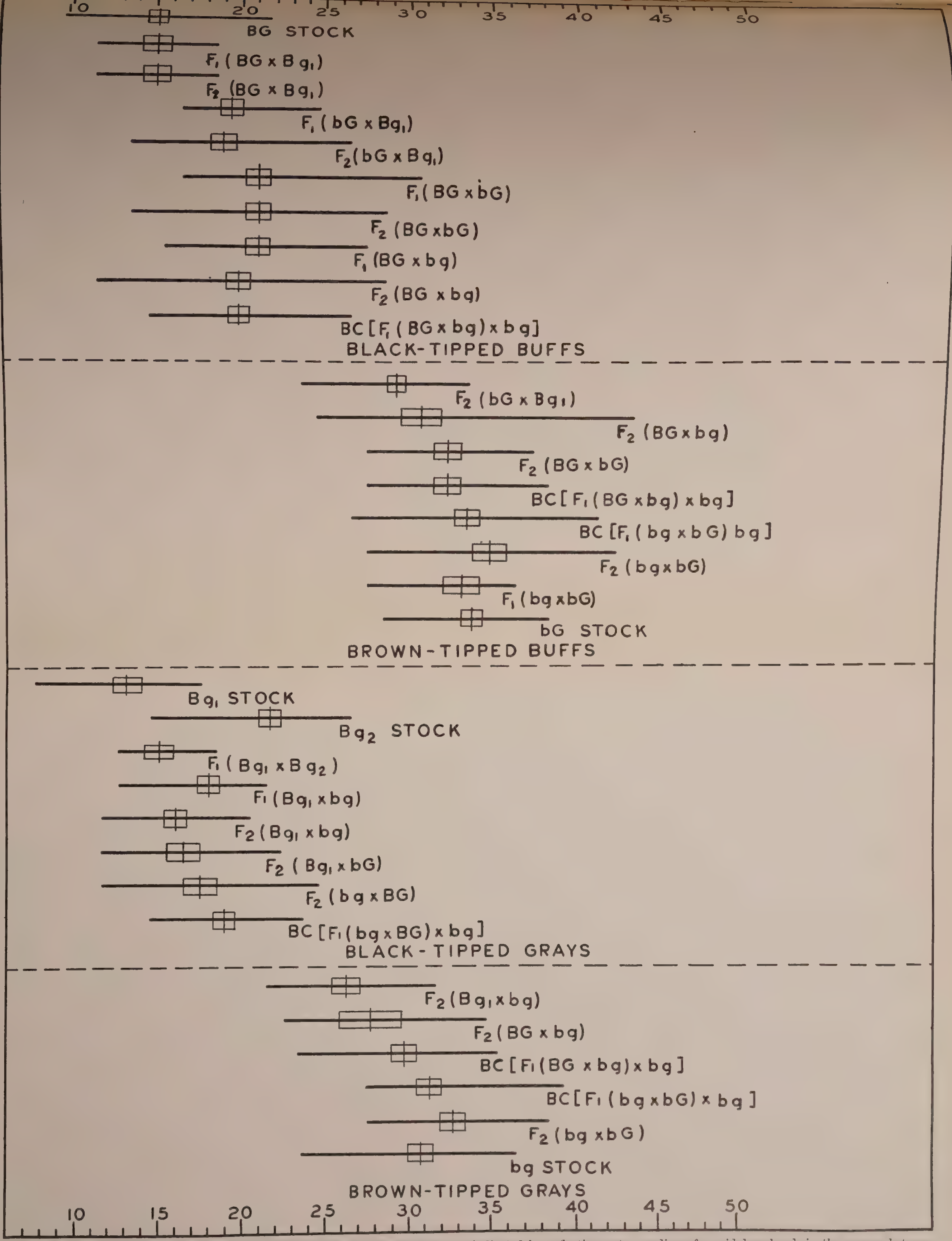

FIG. 1. The mean shade of color of the different classes of segregants, as indicated by reflection-meter readings for mid-dorsal red, in the crosses between *blandus* color strains. The graph is made after the method of Dice and Leraas (1936). The length of each line represents the extremes of that set of measurements. The middle crossbar represents the mean. Two other crossbars are placed two times the standard of the mean above and below the mean, respectively, forming a rectangle. If the rectangles of two comparable lines do not overlap in horizontal position the two means are indicated to differ by a statistically significant amount. Genetic symbols are: black-tipped and brown-tipped (*B b*) and buff and gray (*G g*). A subscript one following the symbol for gray (i.e., g_1) indicates a dark selection line; a subscript two following the symbol for gray (i.e., g_2) indicates a pale selection line.

the F_2 showed no significant deviation from the expected 9 : 3 : 3 : 1 ratio (Table III). Brown-tipped gray and black-tipped buff are recombination classes.

Additional evidence that we are dealing with two pairs of alleles is afforded by the cross between brown-tipped gray and black-tipped buff. This cross produced black-tipped buff offspring. The F_2 generation comprised 110 black-tipped buffs, forty-four black-tipped grays, thirty-eight brown-tipped buffs, and twelve brown-tipped grays. These frequencies showed no significant deviation from the expected 9 : 3 : 3 : 1 ratio (Table III). The brown-tipped buff and black-tipped gray are recombinations.

Some of the F_1 progeny of the cross between brown-tipped gray and black-tipped buff were backcrossed to brown-tipped gray. This backcross affords a test for linkage between the black-tipped, brown-tipped alleles (B b) and the buff, gray (G g) genes. The test is in the coupling relationship. The test-cross generation comprised 237 mice (Table IV). Parental types (black-tipped buff and brown-tipped gray) and recombinations (black-tipped gray and brown-tipped buff) occurred in the progeny in frequencies of 120 and 117, respectively, which do not depart significantly from the 1 : 1 ratio between parental and recombination types that would be expected in the absence of any linkage between the genes (G) and (B).

The black-tipped buff and black-tipped dark gray strains were crossed and produced both black-tipped buff and black-tipped gray offspring, apparently indicative of considerable heterozygosity in the parental buffs. The buff offspring were duller and grayer than the mice of the parental, buff strain. The buff F_1 mice were mated to produce an F_2 generation of thirty-seven individuals (Table III). In this generation it was difficult to separate all of the progeny into buff and gray classes. Eleven mice, or approximately the expected number of homozygous buffs, were unquestionably buff. Nine, or nearly the expected number of homozygous grays, were unquestionably gray. Seventeen, or approximately the expected number of heterozygotes, were difficult to assign to either class. This apparent suppression of buff dominance in the cross between dark strains will be discussed later.

VARIATIONS WITHIN MAJOR COLOR CLASSES

Only a part of the color variation in the Tularosa deer-mice can be attributed to the effects of the major gene pairs (G g) and (B b). Minor variations in saturation and intensity of color permitted selection within the several color strains. Furthermore, the segregants into major color classes in the crosses showed effects of the crosses that must be attributed to other genes than those determining the major color types. The continuous nature of this variation within color strains leads to the conclusion that a relatively large number of genes with minor, additive effects is involved here. Reflection-meter readings of the shade of color of the segregants in the crosses and of the parental stocks (Fig. 1) provide some measure of the effect of these minor genes.

Before considering the minor variations within the major color types, it is well to recall the nature of selection in the parental strains. The black-tipped buff line was selected toward an increased saturation of buff. The black-tipped dark gray strain was selected toward darkening the shade of the pelage color. These two stocks are presumed to carry a majority of the genes for increased intensity of pigment. Black-tipped pale gray mice were selected toward paleness of pelage. The brown-tipped buffs and grays were selected toward paleness, but selection was more rigorous in the gray than in the buff line. These three stocks that were selected toward paleness are presumed to carry a minority of the intensity genes.

Black-tipped gray was the only major phenotype in which selection was attempted toward both paleness and darkness of pelage. The black-tipped dark grays, derived from the selection toward darkness of pelage, average much darker than the black-tipped pale grays obtained by selection for paleness (Table I). The reflection-meter readings for mid-dorsal red in the black-tipped dark gray strain average 12.85 ± 0.32 units, and the readings for the black-tipped pale gray line average 21.60 ± 0.24 units. The difference between the means is 8.75 ± 0.40 units, which is highly significant. The dark gray strain was crossed with the pale gray line, and an F_1 generation was raised. The progeny were intermediate between the parental stocks in shade of pelage, but they were closer to the parental dark grays than to the pale grays (Fig. 1). It seems evident that the black-tipped dark gray line contains a number of genes for increased darkness of shade, while the black-tipped pale gray line has a reduced number of these minor genes.

When black-tipped mice, presumably carrying a number of minor intensifiers, were crossed with brown-tipped mice, which had been selected against these minor genes, the black-tipped segregants in subsequent F_1, F_2, and backcross generations were always paler in shade than the parental black-tipped mice (Fig. 1). Three types of crosses between black-tipped and brown-tipped resulted in a tipped buff F_1 generation. The cross between brown-tipped buff and black-tipped dark gray had less paling effect on the black-tipped buff F_1 mice than did the other two crosses. The crosses between black-tipped buff and brown-tipped buff and between black-tipped buff and brown-tipped gray produced the palest F_1 black-tipped buffs. In the first of these three crosses most of the minor genes for darkness of shade presumably came from the black-tipped dark gray parent, which had been selected intensively toward darkness of pelage. Therefore, it is to be expected that the cross involving this strain would produce a darker F_1 than would the crosses involving the black-tipped buff strain, which had been selected for saturation of buff rather than total darkness of pelage.

Black-tipped buff segregants in the F_2 generations of the above-mentioned three crosses showed no significant differences in shade of pelage from the corresponding F_1 generations. As would be expected, however, the F_2 generations showed increased variability over the F_1 progeny. A backcross of the progeny of the cross, black-tipped buff × brown-tipped gray, to brown-tipped gray reduced the range of variation in shade but did not change the mean. The black-tipped gray segregants in crosses between black-tipped dark gray and the brown-tipped lines were all paler than the parental black-tipped dark gray strain (Fig. 1). In the cross between black-tipped dark gray and brown-tipped gray, the F_2 black-tipped grays were darker in shade than were the F_1 mice, but they averaged paler than the parental black-tipped dark grays.

Brown-tipped segregants in the F_2 generations of crosses between black-tipped and brown-tipped averaged darker than their respective, parental, brown-tipped strains. The darkest of the brown-tipped gray segregants were in the F_2 generation of the cross between brown-tipped gray and black-tipped gray. The darkest of the brown-tipped buffs likewise segregated out in the F_2 of the cross between brown-tipped buff and black-tipped dark gray. This is additional evidence that the dark-tipped dark gray line carries a larger number of the minor intensifiers of shade than does the black-tipped buff strain.

Summing up this section: (1) Black-tipped strains of deer-mice, which had been selected for darkness of shade of pelage color or for saturation of buff, respectively, showed increased paleness of shade and saturation after crossing to brown-tipped strains that had been selected for relatively pale pelage color, (2) the brown-tipped lines showed increased darkness of shade after crossing to the selected, black-tipped lines, (3) these effects are attributed to the action of several, minor genes with additive effects on the intensity of the sepia and yellow-orange pigment.

DISCUSSION

The major differences in pelage color of the Tularosa deer-mice result from the action of two pairs of alleles. Buff and gray mice differ in the pigmentation of the subterminal band of the agouti, or body, hairs. Buff mice have conspicuous yellow-orange pigment in this band, whereas gray mice lack discernible quantities of yellow-orange. The exact physiologic action of these genes (*G g*) is not yet known. Black-tipped and brown-tipped mice differ in the intensity of the sepia pigment in all of the hairs. Black-tipped mice have large black clumps of closely packed sepia granules. Brown-tipped mice have smaller clumps of loosely gathered sepia granules.

Variations within the limits of the major color types appear to result from the effects of an unknown number of genes with minor, additive effects on intensity of pigment. Some of these genes apparently affect only the sepia pigment, and others apparently affect only the yellow-orange. Others possibly affect the intensity of both types of pigment. It is not surprising to find these modifiers of intensity in the deer-mice, for such genes have been long known in such mammals as the house mouse (Grüneberg, 1943) and the guinea pig (Wright, 1942).

The major genes for buff and gray and the minor intensifiers of pigment are principally responsible for the remarkable color variability shown by the species *Peromyscus maniculatus.* The recessive gene, brown-tipped, adds to the potential color variability, but only one homozygote was taken in the wild. The buff and gray genes provide the species with considerable plasticity in regard to hue of pelage. The minor intensifiers provide a wide range of potential variation in shade and saturation of pelage color. The close correlation between the pelage color of deer-mice and the soils inhabited that Dice (1940) found in local populations from widely separated parts of North America requires the plasticity afforded by the minor intensifiers of pigment, in addition to the range of variability permitted by the major genes.

Some of the minor, intensity genes have an interesting effect on the buff phenotype. In wild-caught mice buffs usually cannot be identified as such until the mouse has grown its adult coat of hair. In the black-tipped buff strain, which was selected for saturation of buff color, the buff phenotype can be distinguished from gray as soon as the first coat of hair appears. Some modifying genes, which were apparently lost during selection in the buff strain, presumably delay the deposition of yellow-orange pigment until the growth of the adult pelage. When, as pointed out earlier, these black-tipped buff mice are crossed with black-tipped dark grays, the F_1 buffs are a less bright buff than are the parental buffs. They are duller and grayer in appearance than are mice of the black-tipped buff line, and the buff cannot be distinguished from the gray phenotype until the adult pelage appears. The conclusion seems justified that the gray mice brought into the cross modifiers that had been lost during selection in the buff line.

In the F_2 generation of the cross, black-tipped buff × black-tipped dark gray, the segregation is into three classes with frequencies close to the expected for a case of incomplete dominance (Table III). This departure from the relatively sharp segregation in the other crosses between buff and gray probably results from the action of the minor, modifying genes. The black-tipped dark gray parents presumably brought into this cross a major number of minor sepia intensifiers. The black-tipped buff parents presumably contributed a large number of yellow-orange intensifiers and undoubtedly some sepia modifiers. The yellow-orange pigment seems to be suppressed to some extent in the filial generations of this cross by the effects of the minor, sepia intensifiers. This suppression of the yellow-orange is greatest in the presumed heterozygotes of the F_2 generation, in which a single dosage of the buff gene and presumed recombinations of the minor, sepia intensifiers result in a buffy-gray phenotype. Parallel cases of inability to distinguish between all buff and gray individuals, in dark, wild populations of deer-mice, will be discussed in a later report.

SUMMARY

Color variations in a stock of deer-mice (*Peromyscus maniculatus blandus*) from Tularosa, New Mexico, are accounted for by genes with major and genes with minor effects.

Two pairs of alleles with major effects account for the major color phenotypes in these deer-mice: Buff (G), which is characterized by yellow-orange pigment in the subterminal band of the body hairs, dominates gray (g), which lacks the yellow-orange pigment. Black-tipped (B), in which the sepia pigment appears black or dark brown, dominates brown-tipped (b), in which the sepia pigment is pale brown in appearance.

Minor variations within the major color types appear to be due to the actions of several genes with minor additive effects on the intensity of pigment. Some of these genes apparently affect only the sepia pigment, and others apparently affect only the yellow-orange. Some possibly affect both types of pigment. A major accumulation of minor intensifiers of sepia often suppresses the yellow-orange pigment in buff mice.

LITERATURE CITED

Clark, Frank H.
1938 Inheritance and linkage relations of mutant characters in the deer-mouse, *Peromyscus maniculatus*. Contrib. Lab. Vert. Gen. Univ. Mich., 7: 1–11.

Collins, H. H.
1923 Studies of the pelage phases and the nature and color variations in mice of the genus *Peromyscus*. Journ. Exp. Zool., 38: 45–107, 7 pls., 15 figs.

Dice, Lee R.
1932 Variation in a geographic race of the deer-mouse, *Peromyscus maniculatus bairdii*. Occ. Papers Mus. Zool. Univ. Mich., 239: 1–26, 1 fig.
1933 The inheritance of dichromatism in the deer-mouse, *Peromyscus maniculatus blandus*. Amer. Nat., 67: 571–74.
1940 Ecologic and genetic variability within species of *Peromyscus*. *Ibid.*, 74: 212–21.

Dice, Lee R., and Harold J. Leraas
1936 A graphic method of comparing several sets of measurements. Contrib. Lab. Vert. Gen. Univ. Mich., 3: 1–3, 1 fig.

Grüneberg, Hans
1943 The genetics of the mouse. Cambridge: Cambridge Univ. Press. vii + 412 pp., 43 figs.

Huestis, R. R., and Elizabeth Barto
1934 Brown and silver deer-mice. Journ. Heredity, 25: 219–23, 2 figs.

Osgood, Wilfred H.
1909 Revision of the mice of the American genus *Peromyscus*. North Amer. Fauna, 28: 1–285, 8 pls., 12 figs.

Russell, E. S.
1946 Quantitative histological study of the pigment found in the coat color mutants of the house mouse. I. Variable attributes of the pigment granules. Genetics, 31: 327–46, 3 figs.

SUMNER, F. B., and H. H. COLLINS

1927 Linear and colorimetric measurements of small mammals. Journ. Mammalogy, 8: 177–206, 4 pls.

WRIGHT, SEWALL

1942 The physiological genetics of coat color in the guinea pig. Biol. Symposia, 6: 337–55, 3 figs.

Department of Zoology, University of Texas,
Austin, Texas